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Sudden Climate Change: The Land Surface Record
NASA grant NAG5-1847 (01 January 1992 - 30 June 1993)
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Progress Report: 01 January 1993

This project was conceived in response to NASA's stated goals to expand its studies to global scales, and to focus on problems relevant to climate or other global change. In the Geology Program, recommendations given at the Coolfont congress were replaced by goals of, essentially, specifying initial or boundary conditions for atmospheric models of change. Our research was designed in response to this, but indirectly, for we were concerned that future change was being modeled against a dearth of information on the natural baseline, recorded in the geologic record. We had two specific concerns: 1) there has been a tendency to use a few global records (e.g., sea-floor cores) as proxies for climate change, and 2) there was a tendency to overlook geologic evidence suggesting climate change could be much more sudden than the supposed forcing functions (e.g., orbital changes). In addition, there was a strong tendency to "search where there was light," in this case an understandable focus of study on the glacial interglacial transition during the latest 18 ka. If the global records that we have tell us any one thing with clarity, it is that the next *natural* change in the earth system will be a return to glacial conditions, and it may occur suddenly. At the time this proposal was written, it was not yet clear how sudden such a transition may be. Since then, evidence from the Greenland ice cores has been developed that indicates a temporary (hundreds of years) return to full glacial climate occurred over a brief period of years to a few decades, at the Pleistocene/Holocene boundary. However, the previous period most analogous to the present was the Sangamon interglacial, ~ 115 ka. Any serious effort to understand natural climate change, as the background for anthropogenic change, must include the transition from the Sangamon interglaciation to the Wisconsin glaciation.

As proposed, our research was for three years at \$ 241ka per year, for a geologic and remote-sensing study of the onset of glacial conditions between ~115 and 50 ka. We proposed to study the regionally extensive land surface record, rather than the temporally detailed record provided by stratigraphic cores. This approach is useful because it enables the depiction of fossil weather patterns, but it lacks the resolution and detail of the point studies. We intended to study transitions in desert soil types, rock coatings, and paleo ELA (equilibrium-line altitude) trend surfaces in Asia, western North America, and Australia. However, because of budgetary constraints, only 15% (1.5 years at \$72K) of the funding request was authorized, entailing a drastic downscope of the project. On the other hand, the project was augmented specifically to add a radar study of soil roughness and moisture in Queensland, Australia.

Our rescope of the project left the following projects intact: 1) A remote-sensing study of rock coatings in central Australia, complementing an on-going study of iron oxides. 2) A baseline study of mixing in soils using cosmogenic isotopes. 3) Paleo ELA depression in the Tien Shan, central Asia, compared to western North America. 4) theoretical studies of spectral mixture analysis, necessary for interpretation of the Australian data. 5) the imaging radar study of Queensland. After one year of the project, we have made the following progress:

1) The rock-coating study in central Australia had to be postponed until next spring (1994) because of funding and scheduling problems for the aircraft research program. Presently, TIMS is scheduled to be shipped to Australia and flown on an Australia plane in May or June. We will attempt to be on the ground at that time, although the image data will not arrive until after completion of our project. John Adams is proceeding with a study of iron oxides in grain coatings, and has prepared a paper for publication (see attached preprint).

2) A 2.5-m pit was dug in a previously studied soil developed on a 0.25-Ma marine terrace near Cape Mendocino, California, and sampled for cosmogenic isotopes. The soil profile was disturbed in an especially clear-cut way by bioturbation above 1-m depth. Because cosmic neutron fluxes attenuate at a constant rate below a surface, concentration profiles of ^{36}Cl for an undisturbed soil should decrease exponentially with depth, but for the disturbed soil the profile should be stepped. Samples were prepared, Cl was extracted from quartz grains, and the prepared concentrates were shipped to Purdue for AMS analysis. Unfortunately, instrumental problems at Purdue have delayed even preliminary analysis until this winter.

3) The paleo ELA project in Xinjiang Province of China was a major focus. It was jointly supported by EOS/ASTER funding, and builds on reconnaissance field work undertaken during the summer of 1991. Much of the summer of 1992 was spent in securing the necessary permissions for field research in Xinjiang in September. MSS tapes of the study area were ordered and received, and processed at JPL. Critical literature references were located and copied. Formal permission from GSFC for the China research came only days before departure. Field work was conducted from 2 to 29 September. A great deal of experience was gained in dealing with logistical problems, and most of the scientific goals were met.

The basic strategy of the research was to measure ELAs in different glaciated valleys of the Tien Shan by mapping the distribution of glacial deposits (AAR method) and assigning ages by exposure-age dating using cosmogenic isotopes, and by standard relative-weathering techniques, calibrated on local dated deposits. Participating geologists in addition to Gillespie were R. M. Burke, a noted Quaternary geologist from Humboldt State University, and D. H. Clark, a Ph. D. student at UW. The relative-weathering techniques we sought to use were estimation of soil development on sub-glacial alluvial fans and fan terraces, and visual estimation of boulder surface degradation. Unfortunately, the amount of dated deposits in this part of the world is minimal. Salt weathering of boulders was phenomenal, and

destroyed the basis for comparison with North America, leaving morphometry as the main means of relative age estimation of moraines. Cosmogenic exposure-age dating was problematic because of extensive and deep boulder weathering. Soil development was dominated by influxes of salt, gypsum and calcite. Nevertheless, soil and cosmogenic samples were collected from 25 sites in three different glaciated valleys. Geographic control was by GPS.

It will be possible to conduct joint research with the Xinjiang Institute of Geography along the lines specified in my proposal. Certain of the Chinese colleagues are fluent in English, and the Institute management is eager for the collaboration to continue. However, the facilities in China are not advanced, and most analysis of samples will have to be done in the U.S. This entails a higher financial burden than we had anticipated. Also, necessary air photos were not forthcoming, and topographic data were not very good and were carefully guarded, so that free access was not possible. Because of this, mapping and topographic analysis for field work prior to launch will have to be done using SPOT images - - another added expense. SPOT images of three critical areas were requested from NASA under a separate project funded at UW.

Discussions were undertaken with Peter Molnar to clarify future central-Asian research directions appropriate to NASA and EOS. A presentation of preliminary findings was prepared for the Department of Geological Sciences here at UW.

An application for an importing license for soils was submitted to the USDA. The Department of Forestry already has such license. Sterilization of samples is done by autoclave, following analysis in our case. Instruction on sample preparation were transmitted to our collaborators in Xinjiang. Several loess samples were prepared for isotopic analysis in a study of provenance with graduate student Eric Steig and Prof. Bruce Nelson.

A new Landsat MSS image of the Muzat River in Xinjiang was purchased and analyzed. This image is in the old "band interleaved by pixel pair" format, which we cannot handle yet on my DEC station; therefore, processing was done at JPL, on November 19. This image has a low sun angle and emphasizes the topography of the piedmont moraines well.

4) Theoretical studies of spectral mixture analysis continued at UW. Specifically, more study was directed towards a) hierarchical analysis and b) application to radar and thermal IR images, necessary for interpretation of the Australian data.

5) A new graduate student, Wen Hao Li, was brought on board. His research interests are in TIR and radar remote sensing, and he will work with is in Australia next Fall, under the radar augmentation. The radar overflight scheduled for Fall 1992 was delayed due to structural failure of the NASA DC-8, and had to be postponed a year.

Because of the pattern of equipment failure external to UW, some of the reporting on the results of this study will unavoidably be delayed well past the end of the project in June, 1993.

ROCK WEATHERING IN DESERTS: MOBILIZATION AND CONCENTRATION OF
FERRIC IRON BY MICROORGANISMS

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Submitted to Geomicrobiology Journal
9 January 1992

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Abstract Samples of rock coatings from arid and semi-arid areas in the continental US, Hawaii, and Australia were found to contain siderophores. Pure cultures of most of the bacteria and microcolonial fungi isolated from the same coatings produced hydroxamates and/or catechols. Isolates of common bacteria from the rock coatings concentrated iron oxides/oxyhydroxides on the exteriors of cell walls when exposed to iron-bearing solutions, including siderophores. Siderophores may facilitate mobilization of ferric iron at rock surfaces and at the surfaces of soil grains in desert environments where iron compounds otherwise are relatively insoluble. The experimental loading of bacterial cell walls with iron suggests a natural mechanism for concentrating iron at rock and soil-particle surfaces. Microorganisms provide a means of mobilizing and concentrating ferric iron at aerobic, organic-poor desert surfaces to produce such deposits as rock varnish, iron-stained siliceous crusts and grain coatings, without resorting to special pH conditions.

Key Words: Ferric iron; siderophores, bacteria; microcolonial fungi; rock weathering; soils; rock varnish; deserts

Introduction

Iron oxides and oxyhydroxides are important products of rock weathering and of soil-forming processes. There is an extensive literature, primarily in the soil sciences, that documents the dissolution of primary iron-bearing minerals, the movement of iron in solution, and the precipitation of various iron oxides and oxyhydroxides (Duff et al. 1963; Arrieta and Grez 1971; Birkeland 1984). Less attention has been given to rock

weathering in desert regions, where soils are poorly developed, and where chemical reactions are inhibited by the drier conditions.

Even in humid environments, however, iron in either the ferrous or the ferric states is not readily taken into aqueous solution inorganically under typical pH conditions (Neilands 1974). For example, Neilands points out that the solubility product constant for ferrous hydroxide at 25°C and zero ionic strength is $10^{-15.1}$; for ferric hydroxide it is in the range of 10^{-36} to $10^{-38.7}$. Instead, the iron in most soils appears to be transported primarily by organic chelators (Birkeland 1984; Ugolini 1986), or, in anaerobic, organic-rich environments by dissimilatory reduction of Fe(III) by microorganisms (Lovley 1987; Lovley and Phillips 1988).

Desert surfaces typically are neutral to alkaline, and the amount of organic material at rock surfaces and in shallow soils is low compared to humid regions. Accordingly, conditions are less favorable for mobilization of iron by either inorganic or organic processes. Desert rock surfaces that are not covered by soils are perhaps the least favorable for iron mobility, for they are dry most of the time, largely free of organic materials, and they commonly are exposed to alkaline dust. Even the oxidation of Fe(II) in silicate and oxide minerals at these surfaces is very slow (Colman and Dethier 1986).

Nevertheless, textural and mineralogical evidence suggests that many of the ferric oxide and oxyhydroxide coatings at rock surfaces and on soil particles in deserts have been deposited from aqueous solutions. In warm deserts the most abundant rock coatings are clay-rich desert varnish and silica glaze, which form on exposed surfaces above soils under certain environmental conditions (Potter and Rossman 1977; Dorn and Oberlander 1982; Farr and Adams 1984). Coatings on soil particles include iron oxyhydroxide and clay coats on mineral grains and on desert sands. Ferric-iron stained siliceous crusts are documented from the cold deserts of Antarctica (Friedmann and Weed 1987). If the iron in coatings and crusts is mobilized

and deposited inorganically by aqueous solutions, the source materials essentially are restricted to Fe(II)-bearing materials, owing to the extreme insolubility of Fe(III). Alternatively, Fe(III) could be reduced to Fe(II) by organic acids (Krumbein 1983), or mobilized by chelation, if appropriate organisms are present.

One possible source for the iron in coatings is the Fe(II)-bearing minerals in the host rock or the soil particles. There is abundant evidence, however, that many rock coatings are derived primarily from wind-deposited dust that has been chemically altered under conditions of intermittent wetting and drying at the rock or grain surfaces (e.g. Engle and Sharp 1958; Perry and Adams 1978; Dorn and Oberlander 1982; Farr and Adams 1984; Curtiss et al. 1985). For example, iron-rich coatings occur on quartz, limestone or other substrata that are essentially iron free. In desert varnish iron typically has been concentrated by a factor of 3 to 4 relative to the source dust (Pewe et al. 1981).

Significantly, dust deposits, which may include both local soils and regional materials, are themselves the products of weathering in the oxidizing desert environment, and iron occurs mainly, if not exclusively, as Fe(III) in oxides and oxyhydroxides (Pewe et al. 1981). Thus, relatively soluble Fe(II) compounds may not be readily available in either the dust itself or from mineral grains of the substrata. Alternatively, if Fe(III) can be chelated or complexed organically, there is an abundance of materials available in desert dusts and soils from which to form coatings.

In this paper we explore the hypothesis that ferric iron at rock and grain surfaces in deserts is dissolved by high-affinity biochemical chelating agents, i. e. siderophores, produced by indigenous microorganisms. Furthermore, we investigate a possible mechanism by which microorganisms on rocks could extract iron from solution and accumulate the precipitate on cell walls, and, a similar mechanism by which they may accumulate suspended particles of iron oxides / oxyhydroxides. If

ferric iron can be mobilized and accumulated in this manner it could be concentrated relative to the source dust. To test these hypotheses we have examined rock surfaces and near-surface interiors of rocks from a variety of arid and semi-arid regions. We present additional evidence that microorganisms are present in the outer portions of most desert rocks, and that many types of these microorganisms produce organic chelating compounds that are used to mobilize and concentrate iron. Pure cultures of microorganisms from rock surfaces were exposed to solutions and suspensions containing ferric iron to test for accumulation of iron-oxides on cell walls.

Methods

Methods for culturing, isolating and identifying microcolonial fungi (MCF) and bacteria from rocks were presented in earlier publications (Taylor-George et al. 1983; Palmer et al. 1986; Staley et al. 1992). *In situ* measurements of respiration and photosynthesis were presented in Taylor-George et al.

Hydroxamates in rock-coating extracts were analyzed by a method described by J. B. Neilands using *Arthrobacter flavescens*, American Type Culture Collection (ATCC 1989) strain #29091, which, so far as is known, responds to all hydroxamate siderophores (personal communication). The basal medium was ATCC #424. Weathering coatings on rock samples were removed for analysis with a small grinder. Samples varied considerably in amount, depending on the material available, and included lithobionts when they were present. The surfaces used did not contain epiphytes visible to the unaided eye.

Rock coatings analyzed by us were extracted in 1ml distilled water for 3 days at room temperature with two 1hr heat treatments to 75°C. Extracts were filter

sterilized with a loss of approximately 0.2 ml of sample from retention by the filter. Extracts were concentrated to 0.1 ml by evaporation. Assay plates were prepared as described above, and 20 μ l samples were added to paper disk on the agar surface. The presence of hydroxamate is shown by stimulation of growth of *A. flavescens* around the paper disk after incubation for 20-24 hrs at 30°C. Desferal (desferoxamine mesylate, USP, CIBA) was used as standard at 10 μ g, 1 μ g and 0.1 μ g levels.

Thirteen samples were analyzed by J. B. Neilands, including rock surfaces, MCF scraped from rocks, MCF grown in culture, and fluid from MCF cultures. Solid material to be assayed was sprinkled on basal agar medium previously inoculated with washed suspensions of *A. flavescens*. Liquids from extracted coatings and culture fluids were put on absorbant paper disks on the assay medium. Standards of 10, 20 and 30 pico moles of rhodotorulic acid were used. Both autoclaved and non-autoclaved materials were used.

Methods for analyzing catechols were similar to those used for hydroxamates. The details of the methods were suggested by J. H. Crosa (personal communication). A strain of ENT-7 was used for the assays. Its growth is stimulated by the catechol enterobactin and its precursors, including 2,3-dihydroxybenzoic acid. The assay organism does not respond to all catechols. After growth for 20hrs at 35°C in L-broth cells of ENT-7 were washed twice in saline. A base of M-9CA agar was provided in 50 mm petri dishes. Washed suspensions of ENT-7 were added to M-9CA medium with 0.5% agar and 20 μ M EDDA (ethylene diamine N, N' - diacetic acid) which was pipetted in a thin layer over the base agar. Extracts (20 μ l) were added to paper disks on the agar surface. 2, 3 dihydroxybenzoic acid was used as the standard at 1.0 and 0.1 μ g levels. Plates were incubated at 35°C for 24 hrs.

Bacterial isolates from the surfaces of many of the sample rocks were assayed for siderophores. Streaks of the assay organisms (ENT-7 and *A. flavescens*) were

made on the appropriate media. Streaks of the isolates being tested were made so that they intersected those of the assay organisms. Stimulation of growth of the assay organism in the area of the intersection provided a positive test.

As part of a study of the stability of hydroxamates to autoclaving three hydroxamate producing strains of bacteria isolated from rock coatings were grown for 72 hrs in ATCC 424 broth. The cultures were filtered through 0.22 μm membrane filters. Portions of the filtrates were removed and autoclaved at 121°C for 20 min. Autoclaved and non-autoclaved 50 μl samples were analyzed for hydroxamates by adding them to paper disks on a lawn of the test organism as previously described. Using the same method Desferal solutions were autoclaved at 121°C for 25 min. 10 μl portions, adjusted to provide 1×10^{-5} g, 1×10^{-6} g and 1×10^{-7} g Desferal, were compared with unautoclaved filter-sterilized Desferal. In a similar experiment with the catechol precursor, 2,3-dihydroxybenzoic acid (DHB), autoclaved (121°C for 25 min), and non-autoclaved filter-sterilized solutions, were provided to the assay organism on filter paper disks in amounts of 0.5×10^{-6} g and 1.0×10^{-7} g.

The stability of iron-Desferal and iron DHB complexes exposed to air and daylight were examined by placing 5 ml portions of each in open tubes in ambient laboratory light at 23°C for 3 weeks. Distilled water was added at intervals to maintain volumes. The ratio of Fe to Desferal was 1:25 by weight and of Fe to DHB 1:8, both well within the experimentally determined chelating capacities of the siderophores. After 3 weeks the solutions were examined visually for indications of loss of iron binding.

The ability of some bacteria isolated from rocks to immobilize ferric iron was tested by removing cells grown on K1 agar plates and suspending them in freshly prepared 5 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 0.8% NaCl (with no adjustment of pH) for 10 min. They were then washed 3 times in 0.8% NaCl using centrifugation. Some

preparations were stained with Prussian blue (Feigl 1954; Skerman 1959) and examined at 480x magnification.

Cell suspensions prepared for electron microscopy were fixed with neutralized 3% glutaraldehyde in deionized water and embedded in Medcast (Ted Pella, Redding, CA). Heavy metal fixatives, e.g. osmium tetroxide, and stains, e.g. lead citrate and uranyl acetate, were not used at any stage in specimen preparation. Sections were cut with a diamond knife and examined with a JEOL 100B electron microscope at 60 kv.

Scanning transmission electron microscopy (STEM) was performed as follows: Linescans were collected on the JEOL 100C with Tracor-Northern 30 mm² Si (Li) detector and Tracor-Northern 5500 Analytical System. Areal spectra and images were collected on a JEOL 1200EX with Link 30 mm² detector [Si (Li)] and Link AN10,000 Analytical System. Data on both systems were collected in the STEM mode.

Some of the isolates also were exposed to FeOOH, Fe-Desferal and Fe-DHB, as described for exposure to ferric chloride, above. FeOOH was prepared by adjusting the pH of ferric chloride solution to pH 7 and diluting to 10 mM. The Fe-Desferal complex was produced by adding Desferal to 5mM FeCl₃ · 6H₂O, 1 mg Fe : 24.4 mg Desferal. Fe-DHB was prepared by adding DHB to 5mM FeCl₃ · 6H₂O at 1 mg Fe to 8 mg DHB. In addition, isolates were exposed for 1 hr to FeSO₄ which was prepared at 5 mM. After washing 3 times in 0.8% saline using centrifugation the preparations were stained with Prussian blue and examined.

Results

The bioassay tests described in the above section were used to test for the presence of hydroxamates and catechols in samples of powder that were scraped from the surfaces of rocks having visible coatings of clay-rich varnish or silica glaze. The samples were collected from arid and semi-arid regions in the continental US, Hawaii, Australia and Antarctica. Samples ranged in size from about 10 mg to about 400 mg, depending on the thickness and the area of the coatings that were available.

The bioassay results were positive for siderophores for some of the samples from all localities except Antarctica (Table 1). More of the samples tested positive for catechols than for hydroxamates. Small samples commonly gave negative results. For example, Neilands (personal communication) found no hydroxamates in 10 mg samples of desert varnish from 7 rocks that were supplied by us. Hydroxamates were detected mainly on surfaces where MCF could be seen at 30x magnification. However, surfaces that had abundant MCF or lichen that could be seen without magnification were not used in the tests.

Bacterial isolates from the rocks produced a greater proportion of positive tests than did the rock powders (Table 1). Hydroxamates were detected in 18 of 59 bacterial isolates tested, and catechols in 18 of 29 isolates. Both hydroxamates and catechols were produced by 8 of the bacterial isolates. Many isolates grew poorly or not at all on the catechol assay medium. Where results were variable between runs or between duplicates, the results are not listed. The catechol assay does not detect all catechols, so negative results do not indicate their absence.

Two MCF samples from rock scrapings were positive for hydroxamates. Of four MCF cultures both the cells and the filtrates of two were positive; the cells and filtrates of the other two were negative.

Experiments were conducted to test the stability of siderophores when heated and when exposed to light and air (see Methods section, above). Autoclaving Desferal and DHB at 121°C resulted in significant loss of activity (Table 2). Exposure of iron complexed with Desferal and DHB to light and air produced visible precipitation, and changes from the colors typical of the respective complexes.

The results of the above experiments demonstrate that siderophores are present in surface coatings of rocks from a variety of localities, and that pure cultures of bacteria and fungi isolated from the coatings in many cases can produce siderophores. Positive results are considered definitive. Negative results do not always indicate the absence of siderophores, for the tests were not sensitive to certain catechols, and the tests may not be definitive on small samples. Negative assays for some samples may also reflect the recent conditions at the rock surfaces. The assay itself involves a water extraction, and, because the siderophores are water soluble, those samples that have been subject to recent rain may be depleted. Similarly, siderophores may not persist in rock coatings that have been heated strongly under desert conditions.

Twenty-five bacterial isolates taken from rocks were investigated to test whether the bacteria precipitated Fe(III) on the exterior surfaces of their cells when suspended in a 5 mM solution of ferric chloride. Prussian blue stain was used to test all samples for the presence of iron. Twenty of the isolates stained positively, three stained weakly, and two were negative (Table 3).

Seven of the isolates were sectioned and examined by electron microscopy. Electron micrographs showed an accumulation of dark (electron dense) clumps of material on the exteriors of the cell walls of the (unstained) bacteria that had been exposed to ferric chloride solution (Figure 1). One sample that did not test positive by staining did not show dark accumulations on an EM image.

Thin sections of four of the bacterial strains (85-9B, 85-13B, 87-3B, and 87-8A) were analyzed by transmission electron microscopy and energy-dispersive x-ray methods (STEM-EDAX). EDAX analyses along line-scans transecting the cells confirmed that the extracellular dark deposits were concentrations of iron and this was confirmed by areal scans as well (Figure 2). Although the iron was primarily concentrated external to the cell wall of these bacteria, in some strains iron was detectable in the periplasmic space as well (Fig. 2D). The STEM images appear to show significant iron in the cytoplasm of some cells.

In addition, cells that had been suspended in ferric chloride solution were viewed under an optical microscope at the same time that a hand magnet was moved under the base of the slide. The magnet did not affect the iron deposits on the cells, indicating that the iron-bearing phase was not magnetite or maghemite. Considering that the deposits were formed from Fe(III) in the presence of oxygen it is likely that they consisted of an amorphous Fe(III) oxyhydroxide or ferrihydrite (Beveridge 1989).

The results of exposure of cells to solutions of FeSO_4 , Fe-Desferal, and Fe-DHB and to a precipitate of FeOOH are given in Table 4. Each of the three strains tested positive for Fe(III) after exposure to a solution of FeSO_4 , implying that oxidation of Fe(II) occurred upon precipitation onto the bacterial cells. A positive test was indicated for the three strains after they were suspended in a solution of Fe-DHB. One of the strains tested positive for Fe-Desferal. The bacterial strain that tested positive for both Fe-Desferal and Fe-DHB produces a hydroxamate and a catechol. Two of the three bacterial strains appeared to have adsorbed particles of the FeOOH floculate during a 1hr exposure; however, the results were not conclusive.

Iron Mobilization and Concentration

Siderophores are well known compounds that microorganisms secrete to obtain iron from their environment for metabolic purposes (Neilands 1984; Neilands et al. 1987). These chelating compounds are produced by many of the fungi and by all classes of bacteria except perhaps the lactobacilli (Neilands 1981). The possible role of siderophores in complexing iron and contributing to the etching of rocks in cold and dry environments was pointed out by (Callot et al. 1987). However, no systematic work has been done to test for the presence of siderophores at rock surfaces or to test whether the microorganisms found on the rocks from different areas are able to produce siderophores.

Our results confirm that both hydroxamates and catechols occur in surface scrapings of rocks from a variety of desert environments (Table 1). In addition, many pure cultures of bacteria and MCF from the same rocks produced siderophores (Table 1). These compounds are water soluble and are unstable when heated (Table 2). Thus, it seems unlikely that the siderophores at rock surfaces persist for long periods in environments where there are occasional heavy rains or where the temperatures become high. The fact that siderophores were detected at all in samples from warm deserts implies that these compounds are replenished by the resident microorganisms. The production of siderophores appears to be stimulated in precisely those environments where iron is not otherwise easily accessible in soluble form, although production may proceed in the presence of available iron (Neilands 1981; Neilands et al. 1987). Although we did not measure the quantity of siderophores produced *in situ* by microorganisms in rocks, and the population of microorganisms in desert rocks is low relative to that in humid soils, the desert environment appears to promote the production of a supply of siderophores beyond what is needed at any given time for metabolic processes.

The presence of siderophores and siderophore-producing microorganisms at rock surfaces suggests a mechanism by which ferric iron can be solubilized (Table 1), without invoking anaerobic conditions, an organic-rich environment, or special Eh-pH conditions. Figure 3 outlines pathways by which iron might be processed. At the top of Figure 3 airborne dust from various sources is transported to the rock substrate. The middle and lower parts of the figure depict how iron from the dust might be transported, concentrated and accumulated. Note that any concentration mechanism requires that the residual material from which the iron has been extracted must be removed, presumably by wind or water.

Most of the iron in the dust is considered to be held in mineral particles in either the Fe(II) or Fe(III) states. The Fe(III) state appears to be dominant in desert dust, which is consistent with its primary origin from oxidized surface soils, and with the extremely low solubility of ferric oxides that inhibits reduction under desert surface conditions. Figure 3 indicates that iron-bearing solutions can be derived from either the Fe(II) or the Fe(III) sources; however, solubilizing Fe(III) requires chelation or organic complexing.

In Figure 3 two possible pathways for mobilizing and concentrating ferric iron are shown: 1) by removing it from an aqueous solution (heavy, solid arrows), and 2) by extracting particles from an aqueous suspension (dashed arrows). Our experiments indicate that bacteria isolated from rock coatings can precipitate iron from solution and concentrate it on external cell walls (Tables 3 and 4). These results suggest a mechanism whereby iron oxides/oxyhydroxides could be concentrated at rock surfaces. As discussed above, iron from the deposited dust would be solubilized either as Fe(II) or as chelated Fe(III) and moved to cell walls where it would precipitate and accumulate.

Bacterial cells also adsorb colloidal iron oxide/oxyhydroxide (Alexander 1971; MacRae and Edwards 1972), as is illustrated by the dashed lines in Fig. 3. Our

experiments with FeOOH yielded inconclusive results, and it is not clear whether the bacteria used in this study can scavenge suspended iron-bearing particles effectively. Furthermore, it is questionable whether sub-micron sized suspended particles of iron oxides are readily available from the source dust, because these small particles are not easily dislodged from host grains by gentle washing. It is not sufficient that microorganisms adsorb very small particles of iron oxides that are attached to larger host particles such as clays, for this would not concentrate the iron unless the larger particles were removed from the system by wind or water. Alternatively, some of the particles of iron oxides released from dead cells may be transported in suspension to living cells, providing a secondary means of concentration.

An additional mechanism of iron deposition, not shown in Figure 3 is also possible. Some portions of the siderophore complexes will not reach any bacterial cell. On the basis of our experiments it would be expected that the complex would be disrupted in the presence of light and air, and the released iron precipitated on the rock surface.

Even though the population of microorganisms is low at rock surfaces in deserts there may be sufficient mobilization and concentration of iron to influence the composition of rock coatings. Based on visual estimates the amount of iron on the cells in Fig. 1 exceeds that required for immediate use by the organisms for metabolism, indicating that at least some of the microorganisms studied can accumulate "excess" iron. This is consistent with other microbiological studies that have shown that many types of bacteria and other microorganisms can accumulate large amounts of extracellular iron compounds (Beveridge 1989; Ghiorse 1984). Whether the storage of iron on the types of bacterial cells analyzed here serves one or more purposes is not known. Neilands et al. (1987) and others have pointed out that gathering and storing iron may be advantageous in an environment where little

soluble iron is available. They also note that iron can form tissue-damaging oxygen radicals, which would require regulation of iron uptake at the membrane level. In addition, oxides of iron are strong absorbers of ultraviolet radiation; therefore, the deposition of oxides about the cells of microbes at desert surfaces may protect them from lethal doses of solar radiation.

In rock coatings many of the bacteria may be attached to inorganic particles, especially clay minerals. Upon death and lysis iron-oxyhydroxide particles on cell walls would be released and could be adsorbed to clay or other host particles (Lovley and Phillips 1988), or recycled by chelation and precipitation onto other living cells (Fig. 3). Thus, the accumulation of "excess" iron by bacteria could lead directly to the build-up of iron on clays. Alternatively, it has been shown in laboratory experiments (Ferris et al. 1988) that iron-loaded cell walls of *Bacillus subtilis* resist autolysis by their own hydrolytic enzymes, and that iron-encrusted cell walls are sites for deposition of small silica crystallites that can silicify and preserve cell structures essentially in tact. Either case provides a plausible mechanisms for fixing the accumulated iron oxyhydroxide as an integral part of the silicate materials that comprise the common types of rock coatings.

Acknowledgements We thank J. Neilands for helpful discussions and for analysis of some of the specimens. Research supported by NASA grant NAGW 1319.

Figure Legends

Figure 1. Transmission electron micrographs of thin sections of unstained bacteria showing accumulation of metals by four different bacterial strains isolated from desert varnish. A: strain 87-8A; B: strain 87-3B; C: strain 87-9B; D: strain 85-13B. Note that the accumulation of electron-dense material (identified in Figure 2 as containing iron) occurs primarily outside of the cell wall. However, in some cells (B) there is evidence of accumulation in the periplasmic space as well. The electron-dense material in some strains (C) is attached to fibrillar material suggestive of an extracellular organic matrix about the cells.

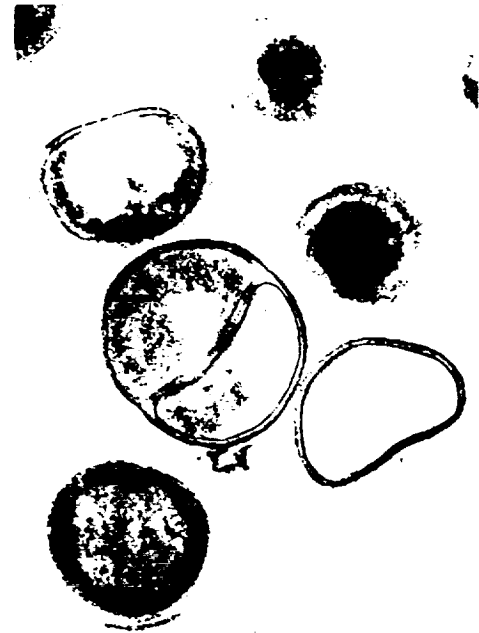
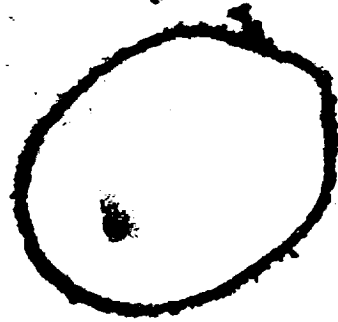
Figure 2. (A) Scanning transmission electron image of bacterial strain 85-9B. (B) Digital x-ray image from the window over the Fe Ka peak. The image has been corrected by subtraction of the bremsstrahlung (background) from the raw integral peak, pixel by pixel. Image is displayed at 0-30% contrast, i.e. the high-iron areas are oversaturated (white) in order to reveal low-concentration areas. Pixel intensities are not proportional to mass fraction.

Figure 3. Pathways for processing iron at rock or grain surfaces in deserts.

Fig. 1



A



B



C



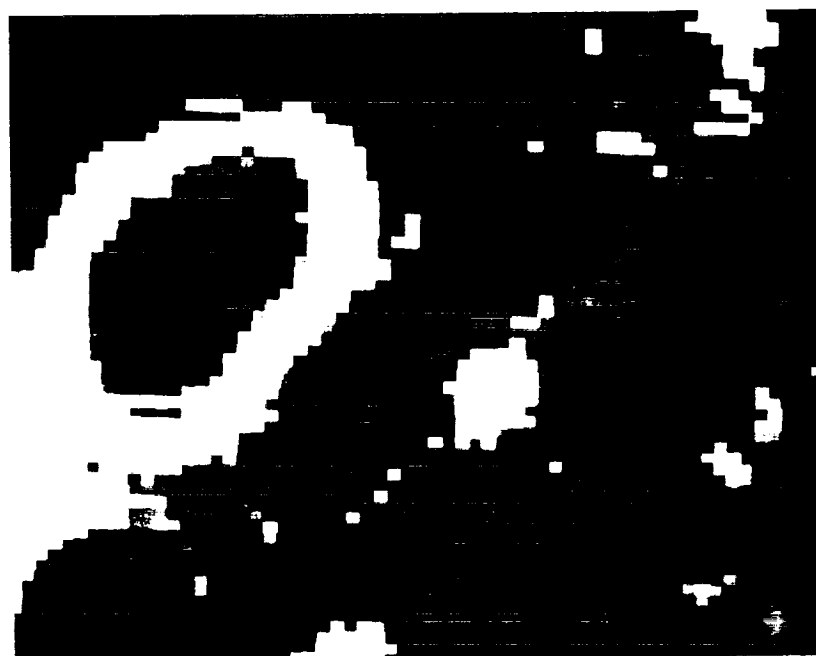
D

1 μ m

Fig. 2



A



B

Fig. 3

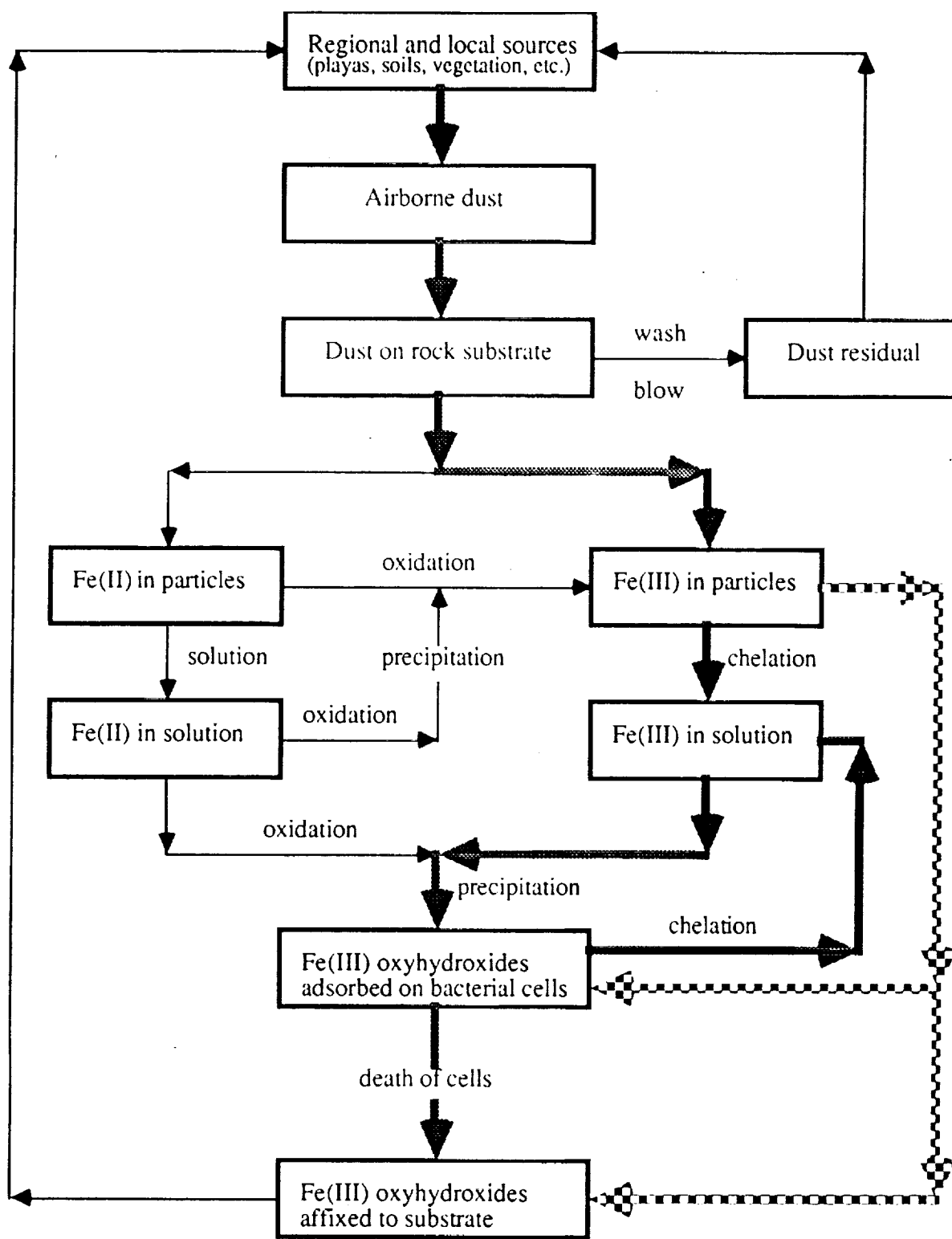


TABLE 1
Siderophores on Rock Surfaces

Rock Samples		Number Positive/Number Tested	
		Hydroxamate ¹	Catechol ²
Australia 1985	silica glaze	1/12	5/18
Australia 1982, 1983	varnish	0/3	1/2
Southwest US	varnish	1/6	6/14
Southwest US	varnish ³	0/7 (10mg)	NA
Hawaii	silica glaze	1/2	1/1
Antarctica	silica glaze	0/2	0/3
Oregon (semi-arid)	varnish	3/5	1/1

Bacteria and MCF from Rocks

Australia 1985	bacteria	9/35	13/18
Southwest US	bacteria	8/17	5/6
Southwest US	MCF ³	2/4	NA
Southwest US	MCF ^{3,4}	2/2	NA
Antarctica	bacteria	1/7	0/5

- 1) Samples considered positive had 0.1 µg or more Desferal equivalent.
- 2) Samples considered positive had 0.1 µg or more 2, 3-dihydroxybenzoic acid.
- 3) Analyzed by J. B. Neilands
- 4) MCF scraped from rock surface

TABLE 2

Heat Stability of Desferal and 2,3-Dihydroxy benzoic acid (DHB)

Sample		Diameter of Stimulated Area on 6 mm Filter Disks	
		<u>Not Autoclaved</u>	<u>Autoclaved</u>
Desferal	0.1 μ g	12 mm	0 mm
Desferal	1.0 μ g	20 mm	12 mm
DHB	0.1 μ g	14 mm	8 mm
DHB	0.5 μ g	40 mm	19 mm
50 μ l Culture Filtrates			
	Strain 85-10B	+	-
	Strain 85-12C	+	-
	Strain 85-13A	+	-

TABLE 3
Concentration of Iron by Bacteria from Ferric Chloride Solution

Isolate	Prussian Blue Test	Electron Microscopy
85-17	+	+
85-9B	+	+
85-13B	+	+
87-3B	very weak	very weak
87-7A	+	+
87-8A	+	+
87-G7C	-	-

TABLE 4

Concentration of Iron by Bacteria from Various Sources
(Prussian Blue Test)

<u>Isolate</u>	<u>FeOOH</u> ¹	<u>FeSO₄</u> ²	<u>Fe-Desferal</u> ²	<u>Fe-DHB</u> ²
85-9B	inc ³	+	inc	+
85-13B	inc	+	+	+ (weak)
87-8A	-	+	-	+

1) Iron provided at 10 mM

2) Iron provided at 5 mM

3) inconclusive

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